

5. Ecology of Hypersaline Microorganisms

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Introduction

The oceans are the largest bodies of saline water with average salinities ranging from 32-35 psu. Hypersaline environments, with salinities far more than the normal seawater salinities, generally originate as a result of evaporation of seawater. Such environments are inhabited by halophiles, the salt loving organisms. Halophiles are distributed in hypersaline environments all over the world, mainly in natural hypersaline brines in arid, coastal and deep sea locations as well as in artificial salterns. Halophiles include prokaryotes and eukaryotes which are adapted to these hypersaline environments at the highest salt concentrations at or close to the solubility limit of NaCl.

Due to the general absence of predators, the halophilic communities are denser in high salt concentration zones. They withstand extreme saline conditions and have the capacity to regulate the osmotic pressure, thereby resisting the denaturing effects of salt in their environment.

The hypersaline microorganisms comprise of a variety of heterotrophic and methanogenic Archaea; photosynthetic lithotrophs and heterotrophic bacteria, as also photosynthetic and heterotrophic eukaryotes. Examples of most widely distributed halophilic microorganisms include archeal *Halobacterium* sp., Cyanobacteria such as *Aphanothece halophytica* and the green algae, *Dunaliella salina* (DasSarma and Arora, 2001).

Halophilism

A common phenomenon in hypersaline environments is the occurrence of gradients in salinity as a result of the evaporation of sea water. Halophiles are characterized based on their requirement of salt for growth in hypersaline conditions. In contrast, halotolerant bacteria do not require NaCl for growth, although they grow in high salinity and in environments devoid of high concentration of salt. Halophiles can be classified into three groups on the basis of their response to NaCl (Ollivier et al., 1994).

1. Slight halophiles which grow optimally at 2-5% NaCl (0.2-0.85 M).
2. The moderate halophiles show rapid growth at 5-20% NaCl (0.85-3.4 M).
3. The extreme halophiles which optimally grow at 20-30% NaCl (3.4-5.1 M).

The non-halophiles grow optimally at less than 2% NaCl (0.2 M). Many halophiles and halotolerant microorganisms can grow over a wide range of salt requirement or salt tolerance at times depending on environmental and nutritional factors.

Characteristics of hypersaline environments

Hypersaline environments are those with salt concentrations above that of sea water (3.3% total dissolved salts). Based on their origin, they can be classified into thalassohaline environments with salt concentration similar to that of sea water: sodium and chloride being the dominating ions and the pH is near neutral to slightly alkaline as these environments result from evaporation of sea water. During evaporation, some changes may occur in the ionic composition out of precipitation of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) or other minerals once their solubility has been exceeded. NaCl saturated thalassohaline brines, such as those found in saltern crystallizer ponds often display a bright red colouration as they harbor a large number of pigmented microorganisms (Oren, 2002).

A great diversity of microbial life is observed in such brines of marine salinity upto 3 - 3.5 M. Hypersaline ponds of Mediterranean salterns (Salins-de-Giraud) in France contain waters with total salinity ranging from 3.4 - 5.1 M (130 - 300 psu) in secondary series and salinities increasing to 340 - 350 psu in the final series where NaCl precipitates in the form of halite crystals (Caumette et al., 1994).

Athalassohaline hypersaline environments in which the ionic composition differs greatly from that of seawater, are likewise populated by microorganisms. A prime example is the Dead Sea, a lake in which the

concentration of divalent cations (about 1.9 M Mg⁺² and 0.4 M Ca⁺²) exceeds that of monovalent cations (1.6 M Na⁺ and 0.14 M K⁺) and of which the pH is relatively low (around 6.0). Even such a hostile environment periodically supports dense microbial blooms (Oren, 1988).

The two largest and best-studied hypersaline lakes are the Great Salt Lake in the Western United States and the Dead Sea, in the Middle East. The Great Salt Lake is the larger of the two (3900 km²) but shallower (10 m) and contains salts that are close in relative proportion to seawater. The Dead Sea, on the other hand is smaller (800 km²) and deeper (340 m) containing a very high concentration of magnesium salts. Both these lakes have close to neutral pH, although the Great Salt Lake is slightly alkaline while the Dead Sea is slightly acidic. Compared to smaller hypersaline ponds, the compositions of these lakes remain fairly constant due to expanse of their size, although recent human activities have had significant effects on the chemistry and biology of both (DasSarma and Arora, 2001).

Wherever seawater penetrates due to seepage through narrow inlets from the sea, small evaporation ponds result. Notable among these are Solar Lake, Gavish Sabka and Ras Muhammad Pool near the Red Sea coast, Guerrero Negro on the Baja California coast, Lake Sivash near the Black Sea, Shark Bay in Western Australia and Ribandar salt pans in Goa, India. Hypersaline evaporation ponds have also been found in Antarctica e.g. Deep Lake, Organic Lake and Lake Suribati, several of which are stratified with respect to salinity.

A number of alkaline hypersaline soda brines also exist, e.g. the Wadi Natrun lakes of Egypt, Lake Magadii in Kenya and the Great Basin lakes of the Western United States (Mono Lake, Owens Lake, Searles Lake and Big Soda Lake), many of which may go intermittently dry. Soda brines lack magnesium and calcium divalent cations because of their low solubility at alkaline pH. Such environments are very dynamic experiencing significant seasonal variations in size, salinity and temperature. In addition to natural hypersaline lakes, numerous artificial solar lakes are man-made for the production of sea salt. Once NaCl precipitates, the concentration of potassium and magnesium chloride and sulfate brines (bitterns) that remain, are usually returned to the sea.

Hypersaline environments also occur in sub-terranean evaporate deposits e.g. the Deep Sea basins created by the evaporation and flooding of ancient seas. Deep Sea brines are relatively stable as a result of their higher density as reported in the Red Sea and Gulf of Mexico (MacDonald et al, 1990). Preliminary studies

have suggested that microbial activity occurs in some Deep Sea hypersaline basins and viable microorganisms have been recoverable from brine inclusions in ancient salt deposits which are over 100 million years old (Vreeland and Powers, 1999).

Adaptations to high and changing salt concentrations

To be able to live at high salt concentrations, halophilic and halotolerant microorganisms must maintain a cytoplasm that is osmotically isotonic with the outside medium. Two different strategies have been used to achieve this osmotic equilibrium. The first option, used by the aerobic halophilic Archaea of the family Halobacteriaceae and the anaerobic halophilic bacteria of the order Haloanaerobiales involves the maintenance of high intracellular ionic concentrations, where K⁺ is the dominant cation instead of Na⁺ and adaptation of the entire intracellular machinery to function in the presence of high salt (Dennis and Schimmin, 1997).

The second option observed in most halophilic and halotolerant representatives of the Bacteria, Eukarya and also in halophilic methanogenic Archaea, involves the maintenance of cytoplasm lower in salt concentrations and the accumulation of 'compatible' osmotic solutes that serve to achieve osmotic equilibrium while not being too inhibitory to enzymatic activity (Gallinski, 1995; Ventosa et al, 1998b). The concentrations of these osmotic solutes are regulated according to the salt concentration in which the cells are found and can be rapidly adjusted as required when the outside salinity is changed. The number of compatible solutes synthesized *de novo* or by accumulation from the medium keeps steadily growing. In either case intracellular sodium concentrations are kept as low as possible and outward-directed sodium pumps in the cytoplasmic membranes are of utmost importance, both in maintaining the proper intracellular ionic environment, and in pH regulation. Adaptation and adaptability of halophilic bacteria depend on the regulation of synthesis of such organic osmolytes as glycine betaine, ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid), glucosylglycerol among others (Galinski and Louis, 1998).

Halocins

Halocins were first isolated by Francisco Rodriguez-Valera and co-workers in 1982. Out of 40 extreme halophiles screened for halocin production, seven

strains inhibited a broad spectrum of strains some 19 – 35 out of 40 strains and 2 strains inhibited 1 – 3 strains.

Halocins are bacteriocins produced by extremely halophilic members of the domain Archaea. Their bactericidal modes of action are wide ranging and include inhibition of transcription, translation, DNA and RNA nuclease activity, pore formation, bacteriolysis and disruption of cellular membranes (Barefoot et al, 1992). Halocin H4 was the first halocin to be characterized and is produced by *Haloferox mediterranei*R4 (ATCC 33500) and was found to be a single protein of about 28 KDa and acted at the membrane level (Messeguer and Rodriguez-Valera, 1985; 1986). Halocin H6, the second halocin to be characterized (Messeguer et al, 1995; Torreblanca et al, 1989; 1990) is produced by *Haloferox gibbonsii* (ATCC 33989). It shares various characteristics with halocin H4, but appears to be more robust as it is insensitive to desalting, trypsin and to heating at 90 °C for 10 minutes. The mode of action appears to be the direct inhibition of the Na⁺/ H⁺ antiporter. The specific target of halocin H4 is still unknown but a possible effect on passive H⁺ permeability on the membrane has been suggested (Messeguer et al, 1995).

Halocin HaLR1: is a microcin and very different from either halocin H4 or H6. It is produced by a partially characterized extreme halophile called 'Halobacterium sp GN101' (GN = Guerrero Negro, Mexico) (Ebert and Goebel, 1985). Hal R1 activity is typical with first activity detected during the transition from exponential to stationary phase. The initial characterization of this halocin was by Rdset and Sturm (1987), with molecular weight of 6.2 KDa, was bacteriostatic and did not lyse nor impart other morphological changes to sensitive cells. Halocin S8 is a second example of a microcin produced by an uncharacterized extremely halophilic rod (strain S8), isolated from salt crystals from the Great Salt Lake (Shand et al, 1999).

Halocins can be easily purified since they are secreted in the environment. They are potential models for a variety of haloarchaeal research. With the exception of H6, their modes of action are unknown and may be able to provide a source of molecules. Thus, the biotechnological implications of halocins are potentially vast but remain unexplored.

Ecological significance of halocins

Halocins are generally produced in the stationary phase. Production of halocins can be advantageous since bacteriolytic halocins can lyse sensitive cells, releasing their contents and enriching the environment.

Bacteriolytic and bacteriostatic halocins, reduce competition when nutrients become abundant again. The halocins take advantage of this nutrient influx in a less competitive environment. Comparative studies have shown at least 15 different types of halocins with different activity spectra. However, no information is available on their ecological existence (Kis Papo and Oren, 2000). Although halocins can be expected to provide competitive advantage to a bacterium that competes with sensitive organisms which being closely related use the environmental resources in almost the same way. Thus a possible role for halocins may be the inter-species competition between different types of halobacteria.

Diversity of hypersaline microorganisms

Halophilic and haloatolerant microorganisms can be found in each of the 3 domains of life: Eukarya, Bacteria and Archaea. There is however a preference to lower optimal and maximal salt concentrations for growth and thus microorganisms are classified according to their requirements and tolerance to salt. The microbial diversity in hypersaline environments have been studied by the analyses of 16S ribosomal RNA genes amplified by PCR from DNA extracted from samples extracted from the environment. Molecular phylogenetic studies indicate a great phylogenetic and physiological diversity of Archaea.

The aerobic halophilic Archaea of the family Halobacteriaceae, order Halobacteriales are the halophiles par excellence and most of the red colourations in saltern crystallizer ponds and hypersaline lakes is due to the C-50 carotenoid pigments found in large concentrations in the membranes of most of the members of this family. The methanogenic branch of Euryarcheota contains halophilic representatives, where methanogenesis can occur upto salt concentrations approaching NaCl saturation. No halophiles have yet been identified within the Crenarcheota kingdom, although they have been isolated.

Within the domain Eukarya, halophiles are scarce. The only microorganism of importance ubiquitously present in high salt environments is the green alga *Dunaliella*. *Dunaliella* is halotolerant rather than truly halophilic and more strains can grow over a broad range of salt concentrations and relatively low concentrations (1 M) can support growth.

The domain Bacteria contains many types of halophilic and halotolerant microorganisms, spread over a large number of phylogenetic groups (Ventosa et al, 1998b). The different branches of the Proteobacteria contain halophilic representatives often having close relatives that are non-halophilic. Similarly, halophiles are also found among the cyanobacteria (Oren, 2000), the Flavobacterium - Cytophaga branch, the Spirochetes and the Actinomycetes. Within the lineages of gram positive bacteria (Firmicutes), halophiles are found both within the aerobic branches (Bacillus and related organisms) as also within the anaerobic branches. In general, it may be stated that most halophiles within the domain bacteria are moderate rather than extreme halophiles. However, there are a few types that resemble the Archaeal halophiles of the family Halobacteriaceae in their salt requirements and tolerance.

Halophilic Prokaryotes

A. Cyanobacteria: are bacterial prokaryotes that are characterized by the presence of chlorophyll a and phycobilin pigments and carrying out oxygenic photosynthesis. They dominate the planktonic biomass and form microbial mats in many hypersaline lakes, a common uni-cellular species *Aphanothece halophytica* grows over a wide range of salt concentration from 2-5 M NaCl and is an extreme halophile with a salt optimum of 3.3 M and lyses in distilled water. It uses glycine-betaine as the major compatible solute which it takes up from the medium or synthesizes it from choline (Galinski et al, 1993). *A. halophytica* has been reported from the Great Salt Lake, Dead Sea, Solar Lake and artificial solar ponds. A planktonic cyanobacterium *Dactylococcopsis salina* is reported from the Great Salt Lake. A variety of filamentous cyanobacteria, e.g. in the order *Oscillatoriales*, such as *Oscillatoria neglecta*, *O. limnetica*, *O. salina* and *Phormidium ambiguum* have also been described in the order *Oscillatoriales*. They develop in the green (second) layer of mats in hypersaline lakes. They are mostly moderate halophiles, growing optimally at 1-2.5 M NaCl and form heterocysts that fix nitrogen. Another common species in the same family is *Microcoleus chthonoplastes*. The

diversity of cyanobacteria occurring in hypersaline environments have not been studied extensively (Caumette et al, 1994).

B. Phototrophic bacteria: Phototrophic bacteria occur beneath the cyanobacterial layers in anaerobic but lighted zones in hypersaline microbial mats. They usually grow anaerobically by anoxygenic photosynthesis and some grow aerobically as heterotrophs. They can use reduced sulphur (hydrogen sulphide, elemental sulphur), organic compounds or hydrogen as electron donors. They include green and purple sulfur and non-sulfur bacteria and are characterized by bacteriochlorophyll pigments. The green sulfur bacteria such as the slight to moderately halophilic *Chlorobium limicola* and *C. phaeobacteriales*, deposit elemental sulfur granules outside their cells and are capable of nitrogen fixation. The moderately halophilic filamentous green non-sulfur bacteria such as *Chloroflexus aurautiacus* are also slightly thermophilic.

Halophilic purple sulfur bacteria such as the Chromatiaceae, which deposit sulfur granules inside cells, include mainly moderate halophiles e.g. *Chromatium glycolicum*, which grows photo-organotrophically using glycolate. *C. violescens* and *C. salexigens* use glycerol. The moderate halophiles *Thiocapsa roseoparsarcina* and *T. halophila* from Guerrero Negro synthesize sucrose and take up glycine- betaine from the environment. The moderately halophilic purple non-sulfur bacterium *Rhodospirillum salexigens* (from evaporated sea water pools), and *R. salinarium* (from a saltern), use glycine- betaine and *R. salexigens* also uses ectoine as an osmolite.

The purple sulfur bacteria *Ectothiorhodospira* sp. dominate alkaline soda lakes in Egypt and Central Africa. The moderate halophile, *E. marismortui* is a strict anaerobe and uses carboxamines as compatible solutes. The extreme halophiles *E. halochloris* isolated from Wadi Natrun was the first bacteria shown to synthesize and accumulate ectoine (Galinski et al, 1985).

C. Sulfur oxidizing bacteria: Below the cyanobacteria and the phototrophic bacteria in microbial mats, halophilic, filamentous carbon dioxide fixing bacteria that oxidize hydrogen sulfide and elemental sulfur to sulfate are found. The filamentous *Achromatium volutans* from Solar lake, *Beggiatoa alba* from Guerrero Negro and *B. leptiformis* from Solar lake are a few examples. A unicellular halophilic, chemoautotrophic sulfur oxidizing bacterium, *Thiobacillus halophilus* from a hypersaline Western Australian lake, has also been reported (Post et al, 1983).

D. Anaerobic bacteria and Archaea: A large variety of facultative and strictly anaerobic bacteria and Archaea inhabit the bottom layers of microbial mat communities and sediments in hypersaline lakes. These include fermentative bacteria, homoacetogenic bacteria and methanogenic Archaea. Fermentative anaerobic bacteria growing at saturated NaCl concentrations, for instance *Haloanaerobacter chitinovorans*, isolated from a saltern is capable of fermenting chitin in brine shrimps and brine flies (DasSarma and Arora, 2001). A moderate halophilic isolate *Haloanaerobacter saccharolytica* ferments carbohydrates. *Halobacteriodes acetoethylicus* from an oil well and *Halocella cellulolytica* which ferments carbohydrates including cellulose. *Sporohalobacter lorretii* and *S. marismortui* are sporogenous and ferment carbohydrates.

Isolation of the anaerobe *Halothermothrix orenii* from a Tunisian salt lake shows that microorganisms may even simultaneously withstand high salt concentration (upto 200 g.L⁻¹) and high temperatures (upto 68 °C), (Cayol et al, 1994).

Several homoacetogens, strict anaerobes that produce acetate from oxidation of sugars or amines viz: *Haloicola saccharolytica*,

Acetohalobium arabaticum have been described. These homoacetogens are likely competitors of Sulfate Reducing Bacteria for hydrogen.

Sulfate reducing bacteria (SRB) use sulfate as the terminal electron acceptor although many SRB can utilize other sulphur compounds, nitrate and fumarate as well. They differ in their ability to oxidize different compounds although most use low molecular weight organic species such as lactate, pyruvate, ethanol and volatile fatty acids or hydrogen as electron donors. A few can use carbon dioxide as the sole carbon source. Some species of SRB are nutritionally more versatile with the ability to grow on high molecular weight fatty acids or simple aromatic compounds such as benzoate, phenol (Postgate, 1984; Widdel, 1988) or alkanes (So and Yang, 1999), toluene (Beller et al, 1992; Edwards et al, 1992), benzene (Edwards and Grbic-Gallic, 1992) and polyaromatic hydrocarbons (Flybridge et al, 1993). Although many slightly halophilic sulphate reducers have been isolated, mostly from marine environments (Loka Bharathi et al, 1991; Llobet-Brossa et al, 2002; Albert et al, 1995), relatively few that survive at an extremely high salinity have been cultured (Table 1).

Table 1: Species of halophilic sulphate reducing bacteria (SRB) isolated from hypersaline environments

Species of SRB	Isolated from	Special feature	Reference
<i>Desulfovibrio gabonensis</i>	African oil pipeline	Strictly anaerobic salinity range from 1-17%	Tardy-Jacquenod et al, 1996
<i>Desulfovibrio salexigens</i>	Dried mud samples E1 Agheila Libya	In chemocline of hypersaline mat	Postgate & Campbell, 1966
<i>Desulfovibrio retbaense</i>	Lake Retba, Senegal	Moderate halophile	Ollivier et al, 1991
<i>Desulfovibrio halophilus</i>	Solar Lake	Grows at 18% NaCl	Caumette et al, 1991a
<i>Desulfobacter halotolerans</i>	Great Salt Lake Utah	Acetate oxidizing	Brandt & Ingvorsen, 1997
<i>Desulfovibrio oxyclinae</i>	Solar Lake, Sinai high salinity	tolerates oxygen and	Krekeler et al, 1997
<i>Desulfocella halophila</i>	Great Salt Lake Utah	Fatty acid oxidizing	Brandt & Ingvorsen, 1999.
<i>Desulfonema</i> sp.	Solar Lake, Sinai Egypt	Light populations	Minz et al, 1999
<i>Desulfomonile</i> sp.	Solar Lake, Sinai Egypt	Non-sporulating salt-requiring strain	Loy et al, 2002
<i>Desulfococcus multivorans</i>	Ribandar salt pans	Organotrophic, prefers formate and grows in hypersaline water and sediment 0-10 cm	Kerkar 2003
<i>Desulfotomaculum</i> sp.	Ribandar salt pans	size 10 mm tolerates upto 24% NaCl	Kerkar 2003

These SRB species can grow at upto 4 M NaCl, albeit slowly. Some species namely *Desulfovibrio salexigens*, *Desulfovibrio desulfuricans*, *Desulfomicrobium baculatus*, *Desulfococcus multivorans*, *Desulfomonile* sp., *Desulfotomaculum nigrificans* have been isolated from the Ribandar salt pans in Goa (Kerker, 2004) and grow at salinities greater than 4.0 M. The osmoregulation of SRB have not been studied extensively; preliminary indications are that they do not synthesize compatible solutes, but accumulate salts internally.

Archaea

Methanogens from hypersaline environments generally use methylotrophic substrates rather than carbon dioxide, acetate and hydrogen and are strict anaerobes. Several highly halophilic methanogens have been identified. They are *Methanohalophilus halophilus* from a microbial mat, *M. muhii* from the Great Salt Lake and *M. portucalensis* from a saltern. The slightly halophile *Methanosalus zhilnac* is also an alkaliphile and a slight thermophile. The extremely halophilic methanogen *Methanohalobium evestigatum* with a NaCl optimum of 4.5 M, is also a thermophile with a temperature optimum of 50 °C. Methanogenesis has also been reported from Deep Sea brine pools in the Gulf of Mexico that contain moderately high salinity, their intra-cellular salt concentration is somewhat higher than that of most bacteria, about 0.6 M KCl but is significantly lower than that for the halophilic Archaea (DasSarma and Arora, 2001).

E. Aerobic and facultatively anaerobic Gram - negative bacteria

Many moderately halophilic, heterotrophic gram-negative bacteria belonging to the Halomonas and Chromohalobacter genera have been described. Other genera with halophilic representative include *Salinivibrio*, *Arhodomonas*, *Dichotomicrobium*, *Pseudomonas*, *Favobacterium*, *Alcaligenes*, *Alteromonas*, *Acinetobacter* and *Spirochaeta* (Ventosa et al, 1998b; Kamekura, 1998). *Arhodomonas aqueoli* has been isolated from a sub-terreanean brine associated from an oil field and capable of nitrate reduction (MacDonald et al, 1990). *Chromohalobacter marismortui* from the Dead Sea also capable of nitrate reduction; *Pseudomonas beijerinckii* from salted beans preserved in brine, *Pseudomonas halophila* from the Great Salt Lake and *Salinivibrio costicola* originally isolated from Australian bacon. Several halomonas sp. are capable of nitrate reduction including *H. elongata* isolated from a solar saltern, *H. halodenitrificans*, isolated from meat-curing

brines and *H. eurihalina* isolated from saline soil, besides nitrate reduction also produces an extra-cellular polysaccharide. Similarly, *H. halodurans* can degrade aromatic compounds. *H. halophila* from saline soil, *H. panteleriense* from alkaline saline soil, which grows at a pH optimum of 9; *H. salina*, from saline soil and *H. subglaciescola*, from beneath the ice of organic lake in Antarctica. These organisms use primarily glycine -betaine and ectoine as the compatible solutes (DasSarma & Arora, 2001).

Amongst Spirocheates, the moderate halophile, *Spirochaeta halophila* found in Solar Lake, is a chemolithotroph capable of iron and manganese oxidation. Amongst the *Flavobacteria*, *Flavobacterium gondwanense* and *F. salegens* are psychrotolerant halophiles isolated from Antarctic lakes.

F. Gram – positive bacteria

This group mainly includes moderately halophilic species in the genera *Halobacillus*, *Bacillus*, *Marinococcus*, *Salinococcus*, *Nesterenkonia* and *Tetragenococcus*. They include cocci such as *Nesterenkonia halobia*, isolated from salterns which produce yellow-red carotenoid pigments; *Tetragenococcus halophilus*, from fermented soy sauces and squid liver sauce, and from brine for curing anchovies, which are capable of lactic acid fermentation. Several *Salinococcus* sp. from salterns have been isolated. Other examples include *B. diposauri* from the nasal cavity of a desert iguana; *B. haloalkaliphilus*, from Wadi Natrun; and *B. denitrificans* from a solar saltern in Southern France; *Halobacillus litoralis* and *H. trueperi* are found in the Great Salt Lake. *Sporosarcina halophila* is an endospore forming bacterium has also been isolated (DasSarma & Arora 2001).

Actinomycetes from saline soils include *Actinopolyspora halophila* which grows best at moderate NaCl concentrations and is one of the few heterotrophic bacteria that can synthesize the compatible solute glycine-betaine and *Norcardopsis halophila*, which uses a hydroxyderivative of ectoine and beta-glutamate as compatible solutes (Ventosa et al, 1998a; b).

G. Halobacteria:

These extreme halophiles grow best at the highest salinities (3.4-5 M) NaCl forming dense blooms (upto 10⁹ cells.ml⁻¹) and resulting in the red colour of many brines. Common species of halobacteria are rods, cocci or disc-shaped, although triangular and even square-shaped species exist. Many are pleiomorphic, especially when the ionic conditions

of the media are altered and most lyse below 1-1.5 M NaCl. Halobacteria are classified as Archaea or Haloarchaea and belong to the family Halobacteriaceae. Eleven genera have been reported, *Halobacterium*, *Haloarcula*, *Halococcus*, *Haloferax*, *Halorubrum*, *Halobaculum*, *Natronobacterium*, *Natronococcus*, *Natrialba* and *Natronomonas* and *Haloterrigena* (DasSarma & Arora, 2001). *Halobacterium* sp. as the first reported genera comprising facultative aerobes require a number of growth factors and slightly elevated temperature (38-45°C) for optimal growth. They are generally amino acid utilizing, possessing distinct features such as gas vesicles, purple membrane and red-orange carotenoids. Many may grow in the absence of oxygen via dissimilatory nitrate reduction and de-nitrification, fermentation of different sugars, break-down of arginine and use of light energy mediated by retinal pigments.

Several species oxidize carbohydrates, e.g. *Haloarcula marismortui*, *H. vallismortis* and *Haloferax volcanii* from the Dead Sea; *Haloferax mediterranei* and *Halorubrum saccharovororum* from salterns; and *Halorubrum lacusprofundi*, a psychro-tolerant species from Deep Lake, Antarctica.

Some halobacterial species growing in alkaline lakes are alkaliphilic and others are acid-tolerant. *Natronobacterium pharaonis* from Wadi Natrun and *Natronococcus occultus* from Lake Magadii have pH optima in the range 9.5-10 and do not grow below pH 5. Slight acidophiles such as *Haloferax volcanii* and *Haloferax mediterranei* grow at pH as low as 4.5.

The intra-cellular salt concentration of salt bacteria has been measured to be extremely high and it is generally assumed that organic compatible solutes are not accumulated in these extreme halophiles. Potassium ions are accumulated upto 5 M concentration. In addition, the content of sodium ions appears in the molar range, although the ratio of cytoplasmic potassium to sodium is high. Proteins of halobacteria are either resistant to salt concentrations or require salts for activity. As a group, they contain an excess ratio of acidic to basic amino acids, a feature likely to be required for activity at high salinity.

A unique feature of halobacteria is the purple membrane, specialized regions of the cell membrane that contain a two-dimensional crystalline lattice of a chromoprotein, bacteriorhodopsin. Bacteriorhodopsin contains a protein moiety (bacterioopsin) and a covalently bound chromophore (retinal) and acts as a light

dependent light membrane proton pump (Krebs and Khorana, 1993). The membrane potential generated can be used to drive ATP synthesis and support a period of phototrophic growth.

Halobacteria produce large quantities of red-orange carotenoids which are necessary for stimulating an active photo-repair system for repair of thymine dimers resulting from ultra-violet radiation. The most abundant carotenoids are C-50 bacterioruberins, although small amounts of beta-carotene and lycopene are also present. Several retinal proteins in addition to bacteriorhodopsin are also produced by halobacteria, one of them is Halorhodopsin, which is an inwardly directed light – driven chloride pump and the other sensory rhodopsins, which mediate the phototactic response (Spudich, 1993). Like many aquatic bacteria, Halobacteria produce buoyant gas vesicles, (DasSarma and Arora, 1997). Gas vesicles are hollow proteinaceous structures surrounding a gas-filled space, which enable the cells to float to more oxygenated surface layers and thus increases the availability of light for purple membrane-mediated photophosphorylation. Halobacteria also contain large dynamic plasmids which are reservoirs or transposable inversion sequences (DasSarma & Arora 2001). This finding has aided the development of genetic tools for analysis and manipulation of these organisms. Halobacteria and methanogens are phylogenetically distinct from bacteria and eukaryotes and are classified as Archaea. They exhibit features characteristic of Archaea, including eukaryotic-like transcription and translation machinery, ether-linked lipids and like some bacteria, a cell-wall S-layer composed of a glycoprotein.

Many representatives of the family Halobacteriaceae (Halobacteria) excrete halophilic bacteriocins (halocins) that inhibit growth of other halobacteria.

Bio-geo chemical processes and adaptability to high and changing salt concentrations

The dense microbial communities occurring in the water body and in the microbial mats of hypersaline lakes often exhibit high activities of photosynthesis, dissimilatory sulfate reduction and other microbial processes, thereby exerting a profound influence on the biogeochemical cycles of carbon, nitrogen, sulfur and other elements (Javor, 1989; Oren, 1998).

Most of the methane formed in anaerobic hypersaline environments is not derived from the conventional methanogenic processes based on reduction of carbon dioxide by hydrogen or by splitting of acetate (energy sources that are more efficiently used by SRB). It rather originates from the breakdown of 'non-competitive' substrates such as methylated amines and dimethyl sulfide. These in turn can be derived from microbial degradation of glycine-betaine, dimethylsulfoniopropionate and other methylated compounds that serve as organic osmotic solutes in many halophilic microorganisms (Oremland and King, 1989; Di Pasquale et al, 1999).

In-depth studies of the sulfur cycle in the hypersaline solar lake (Sinai, Egypt) and Ribandar salt pans (Goa) have shown extremely active sulfate reduction by a varied community of SRB, with spatial, temporal and functional separation of activities among the species

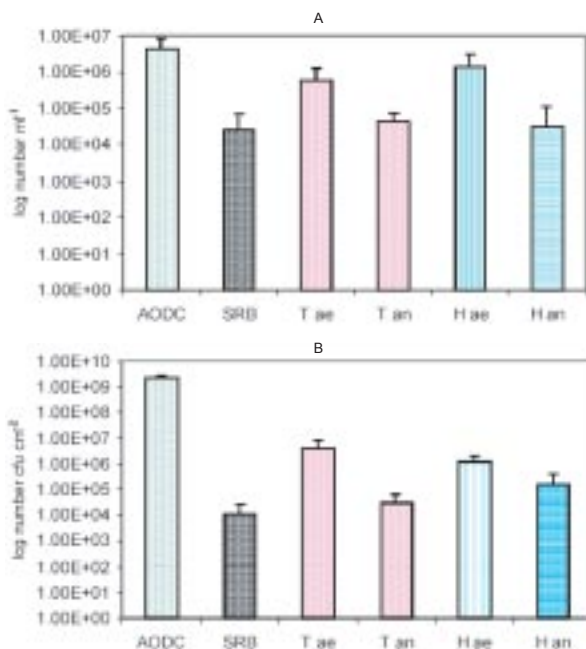


Fig1: The actual number of culturable microorganisms in a thalassohaline salt pan from Ribandar, Goa, India (A): In water, (B): In the top 0-10cm. The total number of bacteria was estimated by acridine orange direct count method (AODC) by Hobbie et al., (1977). The five different groups studied are Sulphate reducing bacteris (SRB), *Thiobacillus denitrificans* like organisms (aerobes T ae; anaerobes T an) and heterotrophic counts (aerobes H ae; anaerobes H an)

present (Kerkar ,2004). The properties of which became established using micro-scale analysis and molecular biological techniques (Loy et al, 2002). In a recent study, it became clear that the abundance of bacterial communities in the highly saline environments is comparable, (Fig. 1) to those found in the normal marine zones. To be able to live at high

salt concentrations, halophilic and halotolerant organisms must maintain a cytoplasm that is osmotically isotonic with the outside medium.

To achieve this osmotic equilibrium two different strategies exist:

1. Maintenance of high intracellular ionic concentrations with K⁺ rather than Na⁺ being the dominant cation and adaptation of the entire intracellular enzymatic machinery to function in the presence of high salt (seen in aerobic halophilic Archaea of family Halobacteriaceae and the anaerobic halophilic Bacteria of the order Haloanaerobiales) (Dennis and Shimmin, 1997).
2. Maintenance of a cytoplasm much lower in salt concentration and the accumulation of 'compatible' osmotic solutes that serve to achieve osmotic equilibrium while not being too inhibitory to enzymatic activity (Galinski, 1995; Ventosa et al, 1998b).

The concentration of these osmotic solutes can be regulated with the changes in salt concentrations where cells are found with respect to the outside salinity (seen in most halophilic and halotolerant representatives of Bacteria, Eukarya and also in halophilic methanogenic Archaeae).

In both the above mechanisms, intracellular sodium concentrations are kept as low as possible and outward-directed sodium pumps in the cytoplasmic membrane are of utmost importance, both in maintaining the proper intracellular ionic environment and in pH regulation (Oren, 1999).

Adaptation and adaptability of halophilic bacteria depend on the regulation of the synthesis of organic osmolytes as glycine-betaine, ectoine, glucosylglycerol and others. Application of molecular biology techniques have led to the insight into the biosynthesis of ectoine in *Marinococcus halophilus* (Galinski, and Louis, 1999) and the production of glucosylglycerol in cyanobacteria of the genus *Synechocystis* (Hagemann et al, 1999). Progress has also been made in the study of the effects of changing salt concentrations on the eukaryotic algae, *Dunaliella* (Gokhman et al, 1999) that accumulates glycerol as the osmotic solute.

Biotechnological aspects

Hypersaline environments are ubiquitous and halophiles can survive in environments that limit the growth of most organisms. Many of the early studies on halophilic microorganisms were initiated in an attempt to understand the bacteria that cause spoilage of salted fish and other salted foods and salted hides. However, halophilic microorganisms

have many positive aspects as well. Halophiles produce a large variety of stable and unique biomolecules that may be useful for practical applications. Pigmented halophilic Archaea and micro-algae absorb light-energy in saltern ponds, thereby raising the water temperature, increasing the rate of evaporation and hastening the deposition of salt. Halophiles possess many hydrolytic enzymes such as DNAses, lipases, amylases, gelatinases and proteases capable of functioning under conditions that lead to precipitation or denaturation of most proteins. Halophilic proteins compete effectively with salts for hydration, a property that may result in resistance to other low water activity such as in the presence of organic solvents. The use of carotenoid rich *Dunaliella* strains for the commercial production of beta carotene for use as health food and food additive (Ben-Amotz and Avron, 1989); and the extraction of ectoine from moderately halophilic bacteria, to be used as enzyme protectant and as a moisturizer in the cosmetic industry (Galinski and Louis, 1998), are some important commercial applications. Novel halophilic bio-molecules may also be used for specialized applications, e.g. bacteriorhodopsin for bio-computing, gas vesicles for bio-engineering floating particles, pigments for food colouring and compatible solutes as stress protectants. Although the current commercial uses of the halophiles are quite significant and many novel and unique properties of many of these organisms, suggest that they have even greater potential for biotechnology (Rodriguez-Valera, 1992).

Conclusion

Hypersaline environments, especially salt marshes are wild and beautiful components of coastal lands. They and their inhabitants make these unique ecosystems fascinating to study. Comparatively little is known as yet about the processes occurring in the salt marshes and their importance to adjacent ecosystems. When examined superficially, they appear rather simple, but a closer investigation reveals tremendous diversity of form and process, which make it unwise to extrapolate the knowledge gained at one site to other marshes.

The enormous diversity of halophilic microorganisms is dispersed over the three domains of life, each with its own interesting and unique properties. There is hardly a hypersaline niche in nature that is not occupied by some halophiles (Oren, 1999). The existing types of Archaea, Bacteria and Eukarya that are able to withstand the stress exerted by salt concentrations up to halite saturations, exhibit a large metabolic diversity that empowers hypersaline ecosystems to function.

Finally, halophiles are an interesting class of extremophilic organisms that have adapted to harsh hypersaline conditions. The diversity of microorganisms in these environments is also of growing interest. The recent findings of bacterial and archaeal metabolic activity suggest that these environments may harbor diverse consortia of microbes that are not easily cultured. Occurrence of novel and stable molecules in halophiles make them valuable for the future biotechnology pursuits.

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